Core practical 1: Investigate a factor affecting the initial rate of reaction

Objectives

- To be able to measure the initial rate of enzyme activity
- To understand why measuring the initial rate is important

Safety

- Trypsin solution at concentrations of 1% or above is an irritant. It may cause an allergic reaction in those allergic to products such as washing powder.
- Wash splashes from the skin as quickly as possible.
- Wear eye protection.

Specification links

- Practical techniques 1, 2, 3, 6 (and 12 if a datalogger is used)
- CPAC 1a, 2a, 2b, 3a–3c, 4a, 4b, 5a

Procedure

Milk protein (casein) is broken down by protease enzymes such as trypsin. The opaque white colour of the milk is replaced by a clear solution. Light passes more easily through the final solution, so the reaction can be monitored using a colorimeter or light sensor.

1. Plan how you will dilute the 1% trypsin stock solution with distilled water to produce additional test solutions of 0.2%, 0.4%, 0.6% and 0.8%. Aim to produce 10 cm^3 of each concentration. Once checked, make up the solutions as planned.
2. Place 2 cm^3 of trypsin solution and 2 cm^3 of distilled water into a cuvette. Use this as a reference cuvette to set the colorimeter absorbance to zero.
3. Measure 2 cm^3 of milk suspension into a second cuvette.
4. Add 2 cm^3 of trypsin solution to the milk in the cuvette. Working quickly, mix and place the solution into the colorimeter and start the stop clock.
5. Measure absorbance immediately and then at 15 second intervals (or more frequently if recording electronically) for 5 minutes, or until there is little change in absorbance.
6. Rinse the cuvette with distilled water and repeat for each concentration.

Notes on procedure

- A check should be done before the lesson to ensure that the milk powder suspension provides an absorbance value within a suitable range when first mixed with the enzyme. Further dilution of the milk may be required, depending on the brand used. Note that absorbance does not have true units, although changes in 'absorbance units' may be discussed.
- Watch that students do not contaminate the stock milk suspension with trypsin as they are transferring solutions to the cuvettes.
- To get repeat measurements when colorimeters are limited, each group can make up one set of concentrations and then pool class results before calculating mean values to use in the absorbance/time graphs.
- Inexpensive simple colorimeters can be constructed using a light-emitting diode (LED), a light-dependent resistor (LDR), a suitable resistor and an ammeter. Various designs can be found using an Internet search.

Answers to questions

1. Independent: trypsin concentration. Dependent: rate of reaction in absorbance units, s^{-1}.
2. Because the reaction is rapid and the milk (substrate) concentration quickly declines. The rate slows as the substrate is used up. Comparisons can only be made at the start of the reaction where controlled variables such as substrate concentration are the same for all levels of the independent variable.
3. A systematic error, because it would cause absorbance readings to be higher than the true value for every measurement.
4. pH – the rate of reaction of enzymes varies with pH, due to changes in the shape of the active site. An enzyme would have the highest rate of reaction at its optimum pH. A buffer might be used to maintain pH at a suitable level.

   Temperature – the rate of reaction of enzymes varies with temperature. As temperature increases, particles gain more energy and more collisions take place between enzyme and substrate particles. Enzymes have an optimum temperature at which the rate of reaction is at its peak. Above that temperature, enzymes will begin to denature, changing the shape of the active site and preventing further catalysis. A water bath and thermometer could be used to maintain a suitable temperature.

Sample data

	Absorbance/absorbance units						
Trypsin concentration (%)	**0 s**	**15 s**	**30 s**	**45 s**	**60 s**	**75 s**	**90 s**
1.0	1.97	1.38	0.68	0.26	0.19	0.16	0.13
0.8	1.97	1.39	0.75	0.23	0.13	0.11	0.09
0.6	1.97	1.42	0.87	0.41	0.14	0.10	0.06
0.4	1.97	1.57	1.21	0.84	0.47	0.30	0.11
0.2	1.97	1.78	1.51	1.28	1.02	0.82	0.58
0.0	1.80	1.80	1.80	1.80	1.80	1.80	1.80

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- Wash splashes of trypsin from the skin as quickly as possible.
- Wear eye protection.
- Inform the teacher if any trypsin gets into your eyes.

All the maths you need

- Recognise and make use of appropriate units in calculations.
- Use an appropriate number of significant figures.
- Construct and interpret frequency tables and diagrams, bar charts and histograms.
- Translate information between graphical, numerical and algebraic forms.
- Plot two variables from experimental or other data.
- Calculate rate of change from a graph showing a linear relationship.
- Draw and use the slope of a tangent to a curve as a measure of rate of change.

Equipment

- skimmed milk powder suspension (2%)
- standard protease (trypsin) solution (1%) IRRITANT
- 6 test tubes and holder
- stop clock
- two 5 cm^3 pipettes (or syringes/measuring cylinders)
- eye protection
- access to colorimeter (see diagram) (or light meter with datalogger)
- 2 cuvettes
- distilled water

Diagram

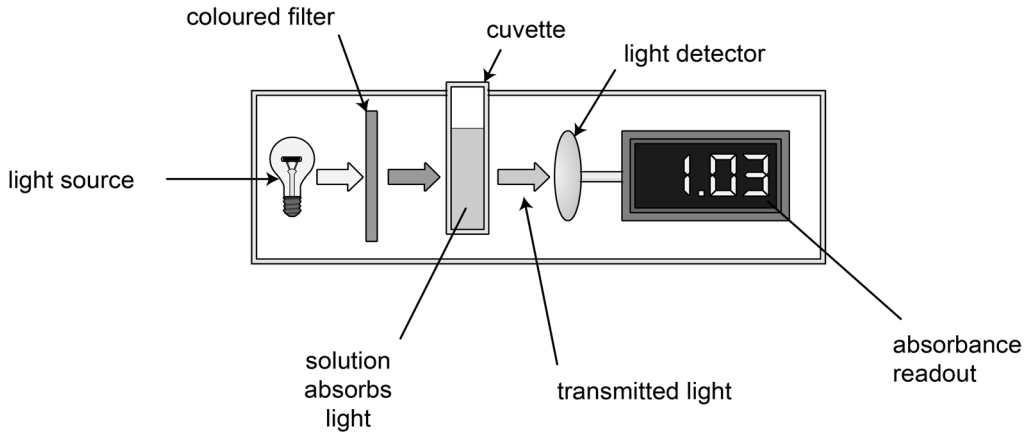

fig A A simple colorimeter.

Procedure

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6. Rinse the cuvette with distilled water and repeat for each concentration.

Analysis of results

1. Record your results in a suitable table.
2. Plot a graph of absorbance against time. It should be possible to plot each concentration as a different line on the same axes.
3. Use the graph to determine the initial rate of reaction for each concentration. Do this by drawing a tangent to the initial part of each curve and calculating the gradient of each line.
4. Draw a second graph to show the initial rate of reaction against the concentration of the enzyme.
5. Write a short conclusion to describe and explain the result of this investigation.

Learning tips

- Use a sharp pencil when drawing graphs. Using different symbols around plotted points will help to distinguish lines when several concentrations are plotted on to one set of axes. Remember to include a key.
- Keep graph scales simple. Using one large square to represent 5, 10 or 20 (or perhaps 0.05, 0.1 or 0.2) is ideal when plotting intermediate points, as the smaller squares will have values that are easy to work with.

Questions

1. What were the independent and dependent variables in this investigation?
2. Why is it important to measure the initial rate of the reaction rather than an average rate over a longer time period?
3. If the surface of the cuvette is scratched, it can result in a greater absorbance of light. If the cuvette used for the reaction was scratched (but the reference cuvette was not), would this give a random or a systematic error? Explain your answer.
4. Suggest two variables that would normally be controlled in enzyme-catalysed reactions but which have not been specifically controlled in this investigation. Explain why they would usually be carefully controlled and suggest how this could be done.

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Safety

- Enzyme powders and concentrated solutions are irritants. Refer to CLEAPSS Hazcard 33 (enzymes).
- They may produce allergic reactions and can be sensitisers (causing allergic reaction on subsequent exposure). This is particularly common in those allergic to products such as washing powder.
- They can cause asthma and can irritate the eyes, nose and skin.
- Avoid skin contact and inhalation.
- Wear disposable gloves and eye protection.
- Use a fume cupboard when handling enzyme powders.
- Wipe up solution spills or any traces of powders with a damp cloth.
- Rinse with plenty of water in case of contact with skin.
- If eyes are contaminated, irrigate for at least 10 minutes and see a doctor.
- Seek medical help if inhalation causes breathing difficulties.

Equipment per student/group	Notes on equipment
skimmed milk powder suspension (2%)	Make up with 2 g of skimmed milk powder in 100 cm^3 water. High fat content milk powders do not give good results. Each student or group will need 5 cm^3 for every concentration tested (30 cm^3 in total if one repeat of each concentration is carried out).
standard protease (trypsin) solution (1%) **IRRITANT**	Mix 1 g trypsin powder in 100 cm^3 water. Add enough alkali (e.g. dilute sodium hydroxide) while mixing it up to produce a pH of 9. When making up the enzyme solution do not heat to a temperature greater than 40 °C to dissolve. Students will dilute this standard solution to give 0.2%, 0.4%, 0.6% and 0.8%. To make up 10 cm^3 of each concentration every student or group needs a total of 30 cm^3.
6 test tubes and holder	
stop clock	
two 5 cm^3 pipettes (or syringes/measuring cylinders)	
eye protection	
access to colorimeter (or light meter with datalogger)	Use the colorimeter with a red filter. Colorimeter output should be sent to a datalogger or computer if possible. CLEAPSS has developed an inexpensive colorimeter that uses paired LEDs. See the CLEAPSS website for details.
2 cuvettes	
distilled water	

Notes